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ON THE INACTIVATION OF STREPTOMYCIN BY ATP-K^+ AND SOME ELECTRONIC ASPECTS OF THIS ACTION

/Following is a translation of an article by Mme Andree Goudot and Michel Paguel, of the Bacteriophage Service of the Pasteur Institute, presented at the 20 May 1963 meeting of the French Academy of Sciences and published in the French-language periodical Comptes rendus de l'Academie des Sciences (Reports of the Academy of Sciences), Vol 256, 1963, pages 5220-5223, under the subject heading of Biochemistry./

The addition of ATP-K^+ to a culture medium containing streptomycin in an inhibitory dose permits the growth of Staphylococcus aureus and of Escherichia coli. An electronic interpretation of this action is given.

We shall describe more in detail, in an article not yet published (this article is to appear in 1963 in Annales de l'Institut Pasteur), the experiments that showed that the addition of ATP-K^+ to complex culture mediums containing sufficient amounts of streptomycin to block the growth of a staphylococcus or of a colibacillus, allowed these two germs, one of them gram-positive, the other one gram-negative, to continue their growth.

We shall only say here that, in these experiments, all our cultures were made in water containing 3% peptone (peptone SC) and 0.3% glucose. Their growth curves were recorded automatically by a M.E.C.I. electronic microbiophotometer (M. Paguel, Ann. Inst. Pasteur, 97, 1959, pp 177-187; La photoelectricite dans l'enregistrement de la croissance bacterienne /Photoelectricity in Recording Bacterial

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Growth7, preface by J. Trefouel, Paris: Herman et Cie., 1941, No 898) and the variations in the growth rate of these cultures were determined by studying these curves.

In this article, we shall study more specifically some theoretic aspects of this action.

Theoretic Results. Streptomycin is composed of three cycles: N-methyl-glucosamine, streptose and streptidine. The N-methylglucosamine and the streptidine are joined to the streptose by some quite labile bonds $H_2C...O...CH$.

In the presence of ATP-K^+, in addition to the decomposition of the streptomycin there is phosphorylation of one or of several of the cycles that compose it. Since the growth of the culture resumes, it is likely that one of the phosphorylated cycles acts as a metabolite, in one of the syntheses necessary for the formation of the bacteria. We made, using electronic chemistry, a theoretic study of the phosphorylation of each of the cycles bound to the streptose, in the presence of ATP-K^+ in the culture medium. We used the molecular orbitales method, in an L.C.A.O. approximation.

1. N-methylglucosamine-K^+-ATP. A calculation of the charges (Fig. 2) shows that the most positive peak is the one found in the streptomycin bound to the streptose, before its dissociation. This peak is the one occupied by the C atom (+0.95) which has the best possibility of being joined by an electrostatic bond to O (-0.94) of the phosphate group. Then we have the following reaction in the culture medium:

$$\text{Streptomycin} + \text{ATP} \xrightarrow{K^+} \text{N-methylglucosamine-PP} + \text{AMP} + \text{streptomycin residue}$$

Is it possible that N-methylglucosamine-PP plays the part of a metabolite in the growth of bacteria? The results of experiments have shown us that the action of ATP-K^+ was stronger, in equal concentrations, for the staphylococcus than for the colibacillus. Staphylococci, gram-positive bacteria, have only a small number of amino acids in their membrane which contains an amino-sugar; muramic acid (M. R. J. Salton, Microbial Cell Walls, New York: J. Wiley, 1960, pp 25-27). This muramic acid forms a metabolite with the aid of uridin-5-pyrophosphate and N-acetylglucosamine. It is also found in gram-negative bacteria, but in a much smaller amount.

This metabolite participates in the synthesis of peptides in the membrane of bacteria. Now, this synthesis takes place on the N-acetylglucosamine cycle. Therefore, N-acetylglucosamine is the active component of the metabolite and it must be assumed, then, that the N-methylglucosamine cycle of streptomycin is what reestablishes growth after its phosphorylation by ATP-K^+.

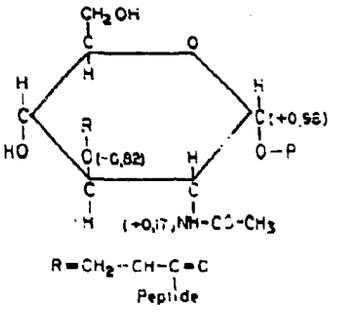

Figure 1

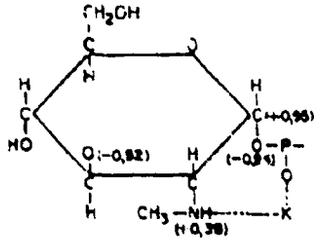

Figure 2

The calculation of the distribution of charges due to the mobile electrons on the muramic acid atoms gives the most positive peak C (+0.95) as united to the uridin-5-phosphate. This peak corresponds to the C (+0.98) peak of the N-methylglucosamine of the streptomycin (Figs. 1 and 2). These two cycles are united, therefore, by the same peak to the uridin-5-phosphate at the time of the synthesis of the

metabolite necessary for the formation of the bacterial membrane.

In addition, this same calculation shows that in muramic acid the peak at which the synthesis of the peptides is the one that has the O (-0.82), that is to say, the one that has the highest negative charge. The same calculation performed on the N-methylglucosamine of the streptomycin shows that the peak occupied by O (-0.92) is the one that has the highest negative charge and that it corresponds by its position to O (-0.82) of the muramic acid, as may be seen in Figures 1 and 2.

2. Streptidine-ATP-K^+. This cycle that is joined to the streptose in the streptomycin has two peaks to which a guanidine /sic; probably should read "guanine"/ group is joined.

They undergo phosphorylation in the presence of ATP-K^+. Without doubt, these guanidine /sic/ groups are the ones used in the E. coli bacterium for the formation of purine again at the time of treatment with streptomycin (H. Roth, H. Amos and B. D. Davis, Biochem. Biophys. Acta, 37, 1960, p 398). Then these purines, produced in excess and not utilized, are rejected along with K^+.

Discussion. What hypotheses can be drawn up according to the results of the experiments and of the calculations produced by the theoretic study? It may be deduced that the N-methylglucosamine cycle is the one that comes into action, after phosphorylation, to permit the growth of the bacteria. Streptomycin releases, in the presence of ATP-K^+, a methylglucosamine-PP that may permit again the synthesis of a muramic acid that is slightly modified in its chemical structure but that performs, nevertheless, the synthesis of the peptides in the same location. The membrane of the daughter bacteria then probably has an amino-sugar in which a methyl group replaces the acetyl group of the muramic acid.

In the absence of ATP-K^+, the peak that should be phosphorylated remains directly united with the streptose.

Therefore, streptomycin acts as an antimetabolite in the synthesis of muramic acid and, because of this, inhibits the formation of the bacterial membrane, whence its inhibitory power on growth.

This fact might explain the necessity of the presence of streptomycin for the growth of certain mutants that should

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have N-methylglucosamine in their membrane. Steptomycin probably provides a metabolite for this mutant.

When the dissociation of N-methylglucosamine occurs in the culture medium by means of phosphorylation, there is a formation of new membranes and no penetration of streptidine. On the other hand, if this phosphorylation takes place in the bacterium after penetration of the streptomycin, there is action by the streptidine which alters the metabolism of the purines which, since they are not produced where synthesis of RNA occurs, prevents the formation of RNA.

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